July 19, 2001

Timothy Adams, Ph.D.
The Flavor and Fragrance High Production Volume Consortia
1620 I Street, N.W.
Washington, D.C. 20006

Dear Dr. Adams:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for the Terpenoid Primary Alcohols and Related Esters, posted on the ChemRTK Web Site on March 20, 2001. I commend The Terpene Consortium of The Flavor and Fragrance High Production Volume Consortia for its commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its Chemical RTK HPV Challenge Program website EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

As noted in the attached Comments, the proposed category approach was in general adequate for the SIDS health endpoints. The test plan for aquatic toxicity provided less explanation for defining these chemicals as a category, and the sponsor needs to consider whether the acetates may not belong in the category for environmental effects.

For physicochemical and environmental fate endpoints, adequate data exist for the purposes of the HPV Challenge Program. However, the submitter needs to provide the input values employed in its EPIWIN calculations and otherwise enhance some of the robust summaries. Similarly for health effects, data are adequate for the purposes of the U.S. HPV Challenge Program but some robust summaries are deficient and need to be enhanced.

The Consortium estimated the transport and distribution of these chemicals using a Level I Model. EPA recommends using the EQC Level III model. For those chemicals having experimental biodegradability or other data available, the sponsor should run the Level III model using experimentally determined values such as biodegradation half-life where available.

EPA agrees with the sponsor's proposal to test geraniol in fish and daphnia. In addition, EPA suggests that geraniol be tested in algae as well because the provided algal test results are inadequate (see detailed comments). EPA disagrees that testing of acetate esters is not necessary, and suggests that geranyl acetate (major component of acetylated myrcene) be tested in fish, daphnia, and green algae (See Test Plan section of Comments).

The Consortium noted that dl-citronellol, geraniol, nerol, and geranyl acetate are on FDA's GRAS list. It may well be, on the basis of experience gained over years of use, that most of the substances have little compelling evidence suggesting that testing is needed in the context of HPV Challenge Program. Nonetheless, while this line of reasoning could have been used to support the recommendations not to test the substances in this category, the information was only provided as background; few examples, and no actual data, were cited.

As with other submissions where the available data are either inadequate or insufficiently documented, this case will remain open until adequate documentation is in hand.

EPA will post this letter and the attached Comments on the Chemical RTK web site within the next few days. As noted in the comments, we ask that the Consortium advise the Agency, within 90 days of the posting on the Chemical RTK website, of any modifications to its submission.

If you have any questions about this response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-260-3470. Submit general questions about the HPV Challenge Program through the Chemical RTK web site comment button or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at tsca-hotline@.epa.gov.

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

/s/

Oscar Hernandez, Director
Risk Assessment Division

Attachment

cc: W. Sanders
A. Abramson
C. Auer
M. E. Weber

EPA Comments on Chemical RTK HPV Challenge Submission: Terpenoid Primary Alcohols and Related Esters

SUMMARY OF EPA COMMENTS

The sponsor, the Terpene Consortium (one of the Flavor and Fragrance High Production Volume Consortia, or FFHPVC), submitted a Test Plan and Robust Summaries to EPA dated February 26, 2001, for the Terpenoid Primary Alcohols and Related Esters. EPA posted the submission on the ChemRTK HPV Challenge Web site on March 20, 2001. The proposed information-gathering plan is for four substances (see Category Definition, below) considered by the sponsor to constitute a category.

EPA has reviewed this submission and has reached the following conclusions:

1. Category Justification: In general, the category approach was adequate for the SIDS health endpoints; the sponsor needs to consider whether, for environmental effects, the acetates may not belong in a category with the alcohols (see Test Plan, below).

2. Physicochemical and Environmental Fate Endpoints: Adequate data exist for these endpoints for the purposes of the HPV Challenge Program. However, the submitter needs to provide the input values employed in its EPIWIN calculations; provide a more detailed reference when referring to the reported values by FMA and provide more information on methods; and clarify the intended use of the robust summary data for the non-category member citral (CAS # 5392-40-5) (see Test Plan, below).

3. Health Effects: Data are adequate for the purposes of the U.S. HPV Challenge Program. However, some robust summaries are deficient and need to be enhanced (see Specific Comments on Robust Summaries).

4. Environmental Effects: EPA agrees with the sponsor's proposal to test geraniol in fish and daphnia. In addition, geraniol should be tested in algae as well because the provided algal test results are inadequate (see detailed comments). EPA disagrees that testing of acetate esters is not necessary, and suggests that geranyl acetate be tested in fish, daphnia, and green algae (see Test Plan, below).

EPA requests that the Sponsor advise the Agency within 60 days of any modifications to its submission.

EPA COMMENTS ON TERPENOID PRIMARY ALCOHOLS AND RELATED ESTERS CATEGORY CHALLENGE SUBMISSION

Category Definition

The category Terpenoid Primary Alcohols and Related Esters contains four predominantly acyclic terpenoid substances that are used extensively in flavors and fragrances. Three of the substances are single chemical alcohols: dl-citronellol, geraniol, and nerol; the remaining substance is acetylated myrcene, a mixture. The principal components of acetylated myrcene are geranyl acetate and neryl acetate (60-65%), limonene (10%), geraniol and nerol (2.5%), and linalyl acetate (2.5%). Except for incomplete identification of minor components of acetylated myrcene, the category definition is clear and unambiguous.

Category Justification

The sponsor justifies this category because of the members' "... close structural relationships and the resulting similarities of their physio-chemical and toxicological properties" (Section 2.2). EPA's comments on each of these criteria follow:

Structural Similarities

The three individual proposed category members (geraniol, nerol, and dl-citronellol) are structurally similar. Geraniol and nerol are primary allylic alcohols, while dl-citronellol is an unsaturated primary alcohol (neither vinylic nor allylic). All of these substances would be expected to undergo similar reactions in the environment, and have similar degradation rates and transport properties. The fourth category member, acetylated myrcene, contains linalyl acetate, a tertiary alcohol, and limonene, a cyclic unsaturated hydrocarbon. Because limonene has no hydrophilic functional groups and it is cyclic, it is expected to have different degradative and transport properties than the other members of this category. Nonetheless, it is a minor component of the mixture, and overall the similarities of the components appear to outweigh the differences.

Similarities in Physicochemical Properties

This criterion is cited in the submission but not discussed.

Toxicological Properties

This criterion is not discussed *per se* in the category analysis, but is mentioned in the discussion of Chemical Reactivity and Metabolism (Section 2.5).

A metabolic chart is provided that depicts the metabolism of geraniol and nerol, and a study (Fischer and Bielig, 1940) is described that indicates that citronellol and geraniol produce similar urinary metabolites. Although these data support the submitter's contention, no quantitative data on the metabolites produced are supplied to assure that all metabolites and metabolic intermediates were identified. Despite limitations in the data available on the metabolism of these compounds, in general it appears that members of this category are likely to undergo similar metabolism.

Other Considerations

To support inclusion of the acetates as category members for environmental effects, the submitter cites a studies of the enzyme carboxylesterase in fish and implies that the primary chemical exposure for fish is thus the hydrolyzed alcohols. EPA believes that this approach is an oversimplification; see the discussion of this point in the Test Plan section below.

The thrust of the category justification was health endpoint-related. Adequate attention should be given to justify the category approach for the other endpoint areas.

Test Plan

Chemistry (melting point, boiling point, vapor pressure, water solubility, and partition coefficient)

Data for these endpoints are adequate for the purposes of the HPV Challenge Program.

Boiling Point: The boiling points for geranyl and neryl acetate are incorrectly reported in the text of the test plan but are correctly reported in the Robust Summaries.

Water Solubility: In the Test Plan, the submitter reports a water solubility value for citronellol of 600 mg/L, and for geraniol a value of 300 mg/L. However the Robust Summaries report values of 300 mg/L for citronellol and 600 mg/L for geraniol. The submitter needs to reconcile these disparities.

Environmental Fate (Photodegradation, Stability in Water, Biodegradation, Fugacity)

Data for these endpoints are adequate for the purposes of the HPV Challenge Program.

The submitter provides photodegradation and biodegradation robust summaries for Citral (CAS # 5392-40-5), a mixture of geranial and neral. The test plan cites only the biodegradation results, without explanation. The reason for including the material should be explicitly stated.

Chemical Transport and Distribution in the Environment: The submitter indicates in its Test Plan that it estimated the transport and fugacity of these chemicals using the Level I Fugacity-based Environmental Equilibrium Partitioning Model. EPA recommends using the EQC Level III model; see Specific Comments on the Robust Summaries. Because some of these chemicals have experimental biodegradability and other data available, in those cases the sponsor should run the Level III fugacity model using the appropriate test results.

Health Effects (acute toxicity, repeat dose toxicity, genetic toxicity, and reproductive/developmental toxicity).

EPA agrees with the overall test plan not to conduct further SIDS testing and has the following comments:

Reproductive Toxicity. The "two-generation" reproductive toxicity study in rats appears to be a one-generation study with an unusual protocol in that only females were exposed to the test material. The fact that male reproductive organs were evaluated in several repeat dose studies with other category members allows this SIDS endpoint to be met for the purposes of the U.S. HPV Challenge Program. In addition, EPA agrees with the submitter's argument that citral (a mixture of geranial and neral) is representative of the category because of the alcohol-to-aldehyde conversion likely to occur in mammals.

Developmental Toxicity. As stated above for reproductive toxicity, EPA agrees with the argument for using the citral data (in this case, appropriately run studies were conducted) to represent the category.

Environmental Effects (Fish, daphnia and algae toxicity)

Geraniol. EPA agrees with the sponsor's proposal to test geraniol in fish and daphnia.

Geranyl acetate. As the test plan states, under environmental conditions the ester hydrolysis half-life is over 23 days, indicating the likelihood of initial aquatic exposure to the parent esters. However, the sponsor avers that testing of esters is unnecessary because of metabolic considerations, arguing (Test Plan, Section 2.5) that the enzyme carboxylesterase exists in fish and therefore the hydrolysis of primary terpenoid acetates to the corresponding alcohols occurs readily in fish. The test plan thus implies that the primary chemical exposure for fish is the corresponding alcohol. This contention is particularly important because esters generally have higher aquatic toxicity than predicted by the baseline narcosis model (ECOSAR, MS-Windows Version 0.99f, USEPA; Lipnick, RL, "A QSAR study of Overton's Data on the Narcosis and Toxicity of Organic Compounds to the Tadpole, *Rana tempraria*." In: ***Aquatic Toxicology and hazard Assessment***: 11th Symposium, GW Suter, II and M Lewis, eds., American Society for Testing and Materials, STP 1007 Philadelphia, PA, 1989, pp. 468-489). EPA believes that the postulated metabolic hydrolysis to the alcohol does not preclude aquatic exposure to the ester. Moreover, it is not known if this enzyme is present in invertebrates, and it is likely not present in algae. Therefore, in order to fully characterize the aquatic toxicity of this category, EPA suggests that geranyl

acetate be tested in fish, daphnia, and green algae. Given the preceding discussion, the sponsor needs to consider whether, for environmental effects, including the esters in this category may not be appropriate.

All aquatic testing should follow the Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD, June 2000-available on the OECD website at http://www.oecd.org./ehs/test/monos.htm).

A number of ECOSAR estimations were included in the robust summaries. These robust summaries were inadequate due to insufficient detail on the data inputs. The sponsor used several measured log P values to predict toxicity instead of calculated log P values as the model used to develop the SAR regression equations. The use of SAR in this manner may give varying results, as illustrated by comparing the predicted and measured aquatic toxicity values submitted for certain chemicals. The sponsor needs to justify using measured log P as an input into the ECOSAR model. The sponsor noted the excess predicted toxicity of geraniol and nerol was due to ECOSAR's treatment of allyl alcohols geraniol and nerol as vinyl alcohols rather than neutral organics. EPA notes that this issue should be adequately addressed by the test results on geraniol.

Fish. EPA agrees with the sponsor's plan to test geraniol using OECD guideline 203 to determine the 96-hour fish LC_{50} and then use the read-across approach for nerol and dl-citronellol. However, EPA also suggests testing of geranyl acetate owing to potential ester toxicity as outlined above. The fish test should be conducted using a closed system with no head space and mean measured concentrations.

Daphnia. EPA agrees with the sponsor's plan to conduct a test on geraniol using OECD guideline 202. EPA also suggests that the sponsor consider testing geranyl acetate for daphnia owing to the ester toxicity rationale already mentioned. The daphnia test should be conducted using a closed system with no head space and mean measured concentrations.

Algae. EPA suggests that the sponsor consider testing geraniol because of inadequacy of reported data and test method. Available studies used an unacceptable test method for green algae (see Specific Comments on the Robust Summaries), yielding results that are qualitative and difficult to associate with the quantitative effects that would be anticipated in the environment. EPA also suggests that the sponsor consider testing geranyl acetate in algae owing to the ester toxicity rationale already mentioned.

Specific Comments on the Robust Summaries

Physicochemical Properties

The boiling point and vapor pressure robust summaries cite "Fragrance Materials Association (FMA) reported values." Furthermore, it is not clear whether the boiling point data provided are measured or calculated. The submitter should provide a more detailed reference for the FMA data and provide method/guideline information.

Water Stability. The submitter cited AOPWIN (which estimates photodegradation) rather than HYDROWIN as the method for estimating the hydrolysis of acetylated myrcene.

Fate

The submitter should provide input values to its Environmental Fate calculations so the data can be adequately evaluated.

All transport and distribution results were estimated using the Level I Fugacity-based Environmental Equilibrium Partitioning Model. EPA recommends the EQC Level III model, which is more realistic and useful for estimating a chemical's fate in the environment, using experimentally determined values such as biodegradation half-life where available.

In order to develop the Level III fugacity model, EPA recommends using the EQC Level III model from the Canadian Environment Modeling Centre at Trent University, which allows full control of data inputs. This model can be found at the following web address: http://www.trentu.ca/academic/aminss/envmodel.

Health Effects

Many of the health endpoint summaries were not considered adequate or were deficient (i.e., need to be enhanced) for the purposes of the U.S. HPV Challenge Program. That some robust summaries are considered inadequate does not mean that new testing is necessary, however, because other existing data with adequate summaries are presented. Specific comments follow:

Acute Toxicity

The respiratory irritation studies in female mice with citronellol, geraniol, and nerol are inadequate because they are not appropriate protocols to assess acute toxicity/lethality. The tests were designed to assess respiratory irritation following exposure periods of one minute.

Genetic Toxicity

Chromosome aberration test in Chinese hamster fibroblast cells with geraniol (Ishidate et al., 1984): There is no rationale for choosing the maximum concentration tested. In addition, there is no rationale presented for not conducting the experiment under metabolic activation conditions. This information is necessary to assess the validity of the test.

Ames test with acetylated myrcene (Mortelmans et al., 1986): Only 70% of the test substance was identified; the identity of the remainder should be provided.

Repeat Dose Toxicity

Twelve week study with citronellol and linalool mixture (Oser, 1958): This robust summary is inadequate because there is no documentation of the relevance of the study design to systemic toxicity. The study was conducted primarily to assess the effects of the test substances on food utilization and the primary endpoints assessed were urinary output of sugar and albumin.

Reproductive Toxicity

"Two-generation" study of citral in rats (Hoberman et al., 1989): As noted above, this study appears to be a one-generation study in which only females were given the test substance. This robust summary is deficient in that the incidence by dose for all effects noted needs to be provided.

Developmental Toxicity

Reproductive/developmental toxicity screening study with geraniol in rats (Vollmuth et al., 1990): This robust summary is deficient because: (1) the test substance was not adequately characterized, and (2) the incidence by dose for all effects noted needs to be provided.

Two citral developmental toxicity studies (Cristina et al., 1995; Gaworski et al., 1992): In both cases, the robust summaries are deficient because the incidence by dose for all effects noted need to be provided.

Environmental Effects

ECOSAR estimates. The sponsor suggests that the ECOSAR estimates for the 96-hour fish LC_{50} values for all substances except dl-citronellol were "overly conservative," while the remainder of the ECOSAR estimates were considered "reliable." The rationale used by the sponsor to determine that the fish toxicity estimates are "overly conservative" is based on one LC_{50} test in sunfish using limonene (an unsaturated cyclic hydrocarbon). No elaboration of the basis for this conclusion was provided in either the Test Plan or the robust summaries.

Algal toxicity. The sponsor reported the results of a set of studies where increasing concentrations of citronellol, geraniol, nerol, and citral were applied to disks placed on agar plates seeded with the alga *Chlorella p.,* then positioned under fluorescent lights for 48 hours. The plates were then examined for inhibitory effects. Interpreting the results of these growth inhibition tests is difficult due to limitations in quantifying the results. In addition, the algorithms used by ECOSAR to estimate algal toxicity were not developed using the inhibition test reported in the Test Plan. Therefore, an analogy cannot be made and geraniol and its acetate should be tested using standard guidelines such as OECD guideline 201. The algal test should be conducted using a closed system with no head space and mean measured concentrations.

Followup Activity

EPA requests that the Sponsor advise the Agency within 60 days of any modifications to its submission.